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13. ABSTRACT (Maximum 200 words) Behavioral, biological, cardiovascular, cellular effects of ultrawide band and high peak power pulsed microwave fields; ocular effects of high peak power pulsed microwave fields, and biological effects of millimeter wave fields were studied using various physiological and behavioral paradigms in animal models. Behavioral studies involved analgesia and open field activity in mice. Cardiovascular effect studies indicated systolic, mean, and diastolic arterial pressures decreased significantly in UWB exposed rats. Cellular effects involved Nitric Oxide Synthase activity in cultured cells, and mutagenic and recombinagenic effects of electromagnetic fields. Millimeter wave studies showed frequency specific neural effects below the effective permissible safety limits. Engineering part of the program involved determination of fields strengths, dosimetry and modeling of radiofrequency fields. A two-step deconvolution routine was developed to characterize ultra fast rise time UWB pulse and has been adopted by other military services to be used in their systems. Details and accomplishments of the research program and a list of publications are presented in this report.					
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## **INTRODUCTION**

The following report summarizes the performance of McKesson BioServices (MBS) staff at Walter Reed Army Institute of Research, US Army Medical Research Detachment, Microwave Bioeffects Branch at Brooks AFB, Texas under the contract DAMD17-94-C-4069.

McKesson BioServices' understanding of technical issues and insight into scientific principles has been the keystone of success for Microwave Bioeffects Branch's research program. Due to our efforts in planning and managing the research program, the research productivity of the Branch has risen dramatically. In the past year alone, dozens of scientific papers have been published or submitted for publication. Moreover, in this period of time our research program has achieved a well-respected stature within the TriService microwave bioeffects arena: our staff participates in international conferences as presenters, our staff has been consulted numerous times by other services and our products are in use in various microwave laboratories.

We are determined to continue to provide high-quality scientific research and to represent the U.S. Army's Microwave Bioeffects program under the guidance of the U.S. Army Medical Research, Development, Acquisition and Logistics Command at Brooks Air Force Base in San Antonio, Texas.

Based on the guidance provided in the contract, McKesson BioServices concentrated its research efforts in the following areas:

- I. Behavioral and biological effects of ultrawide band and high peak power pulsed microwave fields.
- II. Cardiovascular effects of ultrawide band and high peak power pulsed microwave fields
- III. Cellular effects of ultrawide band and high peak power pulsed microwave fields
- IV. Ocular effects of high peak power pulsed microwave fields
- V. Biological effects of millimeter wave fields
- VI. Determination of fields strengths, dosimetry and modeling of radiofrequency fields.

The present report summarizes the efforts of McKesson BioServices staff in the aforementioned research areas and presents a list of publications/presentations made in these fields. In Volume II of this report, you will be able to find the copies of each scientific paper produced by the McKesson BioServices staff during the three base years of the DAMD17-94-C-4069 which covers the time period of May 20, 1994 - May 21, 1997.

## **I. Behavioral and Biological Effects of Ultrawide Band and High Peak Power Pulsed Microwave Fields.**

Based on the following issues: the need for standard for exposure of soldier, increased use of, and incomplete information on biological effects of UWB pulses in and around military sources, Dr Ronald Seaman designed a protocol entitled *"Effects of Ultrawideband (UWB) Electromagnetic Fields on Analgesia and Activity in Mice"*.

The objective of the study was to add information on biological effects of UWB pulses to the database used to set exposure standards for the soldier. Scientists have previously shown that morphine-induced changes are affected by other types of electromagnetic fields. Based on this information, Dr. Seaman tried to determine whether exposure to laboratory UWB pulses changes nociception and activity in mice by using normal nociception and activity as well as morphine-induced decreased nociception (analgesia) and hyperactivity to reach a conclusion. McKesson BioServices' scientific staff exposed animals for 30 minutes to UWB pulses at repetition frequencies of 60/s and 600/s. Appropriate cage and sham-exposed controls were used.

At the above described conditions, normal and morphine-modified activity and nociception were not affected in C57BL/6 mice. In CF-1 mice, normal



nociception and activity and morphine-increased activity were not affected. On the other hand, at 45-minute exposure at 600/s morphine-induced analgesia was possibly affected in CF-1 mice.

It has been observed that UWB pulses changed activity and nociception only for certain conditions in the study; morphine-induced change in nociception in CF-1 mice had been observed after 30 minute exposure at 600/s. Thus, the studies point toward only weak effect on a behavioral endpoint that involves activation of opioid receptors.

Furthermore, to determine whether exposure to laboratory UWB pulses can cause or modify a startle response in rats, Dr. Seaman designed a study to investigate possible UWB pulse modification of acoustic and tactile startle responses, both of which are a rapid neuromuscular response to sudden intense stimuli. The well-studied rat startle response is sensitive to various agents, including microwave pulses. The approach includes: the use of controlled single pulses and brief patterns of pulses, the use of timing of UWB pulses as an independent variable, and the use of appropriate no-pulse trials as controls.

A custom-built startle sensor has been constructed for use in the UWB exposure system by the technical staff of McKesson BioServices. This challenging task has been accomplished during the last months of the contract and the system

is ready to carry out the study described in the approved protocol for UWB pulses.

#### **Relevant Publications/Presentations**

Seaman, R. L., Belt, M. L., Doyle, J. M., & Henry, P. J. (1997). Ultra-Wide-Band Pulses Fail To Modify Morphine-Induced Analgesia and Hyperactivity in Mice. Presented at *The Second World Congress for Electricity and Magnetism in Biology and Medicine*.

Seaman, R. L., Belt, M. L., & Henry, P. (1996). Early Results From a Study Of Ultra-Wide Band Pulses and Morphine Induced Analgesia in the Mouse. *The Eighteenth Annual Meeting of The Bioelectromagnetics Society*.

Seaman, R. L., Belt, M. L., & Henry, P. (1996). Testing For Effects of Ultra-Wide-Band Pulses on Nociception and Activity. *Second CERT Symposium for Environmental Radiation Toxicology*.

## **II. Cardiovascular Effects of Ultrawide Band and High Peak Power Pulsed Microwave Fields**

Dr. Shin-Tsu Lu, as the principal investigator, with the collaboration of Dr. Satnam Mathur, Michelle Belt and Joanne Doyle studied the "*Delayed Cardiovascular Disturbances Determined by a Non-Invasive Procedure in Rats Exposed to Ultra-Wide Band (UWB) Electromagnetic Fields*"

The safety aspect of the ultrashort/ultra-wide band (UWB) electromagnetic field has been widely questioned recently. The central issue is the lack of an adequate biological database to support the UWB Interim Guidelines and modeling for delayed effects of electromagnetic fields in general.

The objectives of this study were:

a) the evaluation of the heart rate (systolic, mean and diastolic pressures) for 4 weeks in Wistar-Kyoto rats exposed to various "doses" of UWB fields by a non-invasive tail cuff procedure without the use of anesthetic agent or tranquilizer.

b) the evaluation of the heart rate (systolic, mean and diastolic pressures) for 4 weeks in Spontaneously Hypertensive rats exposed to various "doses" of UWB field by a non-invasive tail cuff procedure without the use of anesthetic agent or tranquilizer to study the possibility of enhanced sensitivity to UWB

electromagnetic fields due to a common disease.

The uncompensated bradycardia and hypotension can lead to fatigue, decrement in work performance and reduction in brain perfusion. This results in central nervous system effects such as headache, irritability and fainting. Finally, prolonged and untreated hypertension leads to increased risk of cardiovascular accidents. These conditions have serious health implications for the warfighters.

In this study, the animal model used to study the cardiovascular effects of ultrawide band were male Wistar-Kyoto normotensive rats (WKY) and male Spontaneously Hypertensive rats (SHR) of 10-12 weeks of age.

Exposure conditions (six minutes), UWB pulse shapes, and field strengths were:

- sham exposure ( $0 \text{ mW/cm}^2$ )
- UWB, 1 kHz repetition rate, 85 kV/m peak, 1 ns pulsewidth ( $1.95 \text{ mW/cm}^2$ )
- UWB, 500 Hz repetition rate, 93 kV/m peak, 1 ns pulsewidth ( $1.15 \text{ mW/cm}^2$ )
- UWB, 250 Hz repetition rate, 95 kV/m peak, 1 ns pulsewidth ( $0.60 \text{ mW/cm}^2$ )
- UWB, 125 Hz repetition rate, 95 kV/m peak, 1 ns pulsewidth ( $0.30 \text{ mW/cm}^2$ )

The biological endpoints studied were heart rate, systolic arterial pressure, mean arterial pressure, and diastolic arterial pressure. Readings for these endpoints were recorded at the pre-exposure baseline, 1 hour, 24 hours, 72 hours, 1 week, 2 weeks, 3 weeks, and 4 weeks after exposure.

The results indicated that the heart rate in UWB-exposed WKY rats was not altered significantly. However, systolic, mean and diastolic arterial pressures decreased significantly in UWB exposed WKY rats at 1 kHz (1.95 mW/cm<sup>2</sup>) and 500 Hz (1.15 mW/cm<sup>2</sup>) but not at 250 Hz (0.60 mW/cm<sup>2</sup>) or 125 Hz (0.30 mW/cm<sup>2</sup>). Thus, the UWB-induced hypotensive effects could appear as early as one hour after exposure and it could persist throughout the entire study period, 4 weeks after exposure.

Dr. Ronald Seaman also studied *"Ultra Wide Band Pulses: Effects on the Cardiovascular System of the Rat"* as a principal investigator. The increased use of UWB pulses in military systems, the need for standard for exposure of soldier to UWB pulses and the incomplete information on biological effects of UWB pulses were the underlying reasons for this study.

The objective of this research was to add information on biological effects of UWB pulses to the database used to set exposure standards for the soldier. Unlike the previous experiment, this research project was designed to determine whether

acute exposure to laboratory UWB pulses can cause changes in blood pressure and heart rate of the rat.

Briefly this study used animals under ketamine for study of acute effects. Animals were exposed to brief patterns of UWB pulses, lasting 2 minutes maximum. Single UWB exposure of two minutes with pulses at repetition frequencies of 500/s or 1000/s were used as exposure conditions. Aortic catheter and, in some cases, leads for EKG have been used to collect the data. A computerized system has been installed to acquire, record, and analyze blood pressure and EKG in more detail.

No change in mean blood pressure and average heart rate was seen in earlier acute UWB experiments; however, as indicated in a previous study, reduced blood pressure without change in heart rate was seen in Dr. Lu's chronic experiment.

In this experiment, no change occurred in mean blood pressure or in heart rate for animals in acute experiment with 2-minute exposure to UWB pulses at 50/s, 500/s, and 1000/s, confirming earlier acute experiment results.

#### **Relevant Presentation**

Lu, S.-T., Henry, P., & Mathur, S. P. (1996). Manifestation of an Ultra-Wide Band Induced Hypotension in Rats. *Third Annual Michaelson Research Conference*.

### **III. Cellular Effects of Ultrawide Band and High Peak Power Microwave Fields.**

#### **A. Effects of Ultrawideband (UWB) Electromagnetic Fields on Nitric Oxide Synthase (NOS) Activity in Cultured Cells**

NOS has been implicated in other studies of electromagnetic fields in the hippocampus and in peripheral blood vessels. In order to determine whether exposure to laboratory UWB pulses can cause a change in the production of nitric oxide (NO) by cultured cells, established RAW 264.7 cell line and selected transfectants, all of which produce NO through NOS activity have been used. Cell cultures were exposed for 30 minutes to UWB pulses at repetition frequency of 600/s. twenty-four hours later, nitrite concentration, as indicator of NO production, was measured. Appropriate sham-exposed cells were used as controls.

The objective of this study was to add information on biological effects of UWB pulses to the working database used and to set exposure standards for the soldier.

The results indicated that NO production increased in UWB-exposed RAW cells stimulated with gamma-interferon (gIFN), lipopolysaccharide (LPS), and 39 mM nitrate relative to the respective sham-exposed controls. NO production in the RAW cells that were unstimulated and stimulated by gIFN and LPS but not nitrate

was unchanged. NO production in three RAW transfectants that were unstimulated or stimulated in either way was also unchanged. And finally, increased NO production could have resulted from increased activity of NOS, from increased induction of NOS, or from both.

Future research may include: pursuing the investigation of the observed increase of NO production in RAW 264.7 cells, and testing for important UWB field parameters, exposure duration and timing relative to cell stimulation.

#### **B. Mutagenic and Recombinagenic Effects of High-Peak-Power Electromagnetic Pulses**

Genome rearrangements may lead to cell death, heritable mutations, and cancer induction. Studying of genetic effects is obligatory for assessment of any potentially hazardous environmental factor and possible health hazards to exposed personnel. At the present time, no experimental data are available on possible genetic effects of high-peak-power electromagnetic pulses per se or in combination with chemical or physical mutagens.

The objective of this study was to analyze whether high-peak-power pulses might modify spontaneous or ultraviolet-(UV) induced mutagenesis and recombinagenesis.



**Methods:** D7 strain of yeast *Saccharomyces cerevisiae* was used to determine:

- mutations, including reversions, point mutations, deletions, aneuploidy, etc.,
- intragenic mitotic recombination (gene conversion),
- intergenic mitotic recombination (crossing-over),
- colony forming ability (CFA)

Two different microwave sources were used for this study. The UWB exposure system was operated for 30 min at 16 Hz or 600 Hz. On the second series of experiment TEMPO exposure system was operated at 10 and 100 ps (1ps/7s) at 170-180 kW/cm<sup>2</sup> power density. UV 254 nm (100 J/m<sup>2</sup>) was used as a mutagen.

The results indicated that UWB pulses did not affect CFA or induced any mutations or recombinations. UWB exposure did not modify mutagenic or recombinagenic effectiveness of UV. TEMPO pulses did not induce mutations or recombinations. And, exposure to 100 TEMPO pulses decreased CFA in 50% of experiments ( $p < 0.05$ ).

Thus, electromagnetic radiation from UWB and TEMPO sources is unlikely to pose any immediate genetic hazard. The decrease of CFA after treatment with

100 TEMPO pulses appears to result from mechanisms other than chromosome damage (e.g., membrane alterations).

### **C. Effects of Ultra Wide Band (UWB) and Other Pulsed Electromagnetic Fields on Melanosomes from Retinal Pigmented Epithelium.**

The increased use of UWB pulses and other electromagnetic fields in military systems, the need for standard for exposure of soldier to pulsed fields, an incomplete information on biological effects of pulsed fields, and the questions that have been raised concerning retinal effects of pulsed fields are the issues surrounding this area of research.

The objective was to add information on biological effects of UWB and other electromagnetic pulses to the database used to set exposure standards for the soldier and to develop techniques examining the role of melanosomes in reported ocular effects of pulsed microwaves.

The approach was to determine whether exposure to laboratory electromagnetic pulses can cause leakage of free radicals or melanin from melanosomes. In order to achieve this goal melanosomes from bovine retinal pigmented epithelium were used and melanosome preparation have been exposed to electromagnetic pulses such as:

- UWB pulses at 600/s for 30 minutes
- L-band microwave pulses (1250 MHz) at 1/s for 15 minutes
- 200 Tempo pulses over 25 minutes

NADPH oxidation was used as an indicator of melanin radical concentration in medium after exposure. The action by microwave pulses on melanosomes has been suggested as a mechanism for retinal damage.

The results indicated weak or no effect after exposure to UWB pulses. Thus, action on melanosomes, or the melanin they contain, may not participate in mediating effects of RF/microwave pulses on the retina.

#### **D. Electroporation of Cells by High-Peak Power Microwave Pulses.**

Electroporation of cell membrane is a well-known biological phenomena, which has been used for years in biology and medicine to alter the cell membrane permeability to transport substances (drugs, genes, dyes, etc.) into or out of the cell. There are theoretical predictions that high-peak power microwave pulses can alter permeability of cell membrane. A hypothesis has been put forward that electroporation (EP) of membranes could be a possible mechanism of biological effects in FPS-7 and TEMPO exposures.

The objectives were to establish whether electroporation of cells can

be produced by high-peak power microwave pulses (HPPMP), and to determine the threshold conditions for this effect.

The method and procedures for this approach have been tested and are ready to begin systematic experiments with irradiation in 1998. If we show experimentally that EP can be caused by HPPMP, this will be the first observation of EP at microwave frequencies. This will provide with sound explanation of biophysical mechanisms responsible for HPPMP physiological and behavioral effects, and is essential for development of well-founded safety guidelines for HPPMP exposures.

#### **Relevant Presentations/ Publications**

Glickman, R., Seaman, R. L., & Belt, M. L. (1996). Ultra-Wide-Band Pulses Do Not Induce Free Radicals in Melanosomes From Bovine Retinal Pigmented Epithelium. *Second CERT Symposium on Environmental Radiation Toxicology*.

Kiel, J. L., Parker, J. E., Seaman, R. L., & Mathur, S. P. (1997). Engineered Bacteria as Sensors of Weak Ultra-Wide-Band (UWB) Radiofrequency Radiation (RFR) Effects. Presented at *Seventy Third Annual Meeting of the American Association for the Advancement of Science*.

Pakhomova, O. N., Belt, M. L., Belt, M. E., Cox, D. D., & Akyel, Y. (1997). Effect

of High-Peak-Power Pulsed Microwave Radiation on the Colony-forming Ability, Mutagenesis and Recombinagenesis in Yeast. Presented at *The Second World Congress for Electricity and Magnetism in Biology and Medicine*.

Pakhomova, O. N., Belt, M. L., Mathur, S. P., & Akyel, Y. (1997). Effect of Ultrawide Band Electromagnetic Pulses on Ultraviolet-Induced Mutation and Mitotic Recombination in Yeast. Presented at the *Ninth International Congress on Genes, Gene Families, and Isozymes*.

Pakhomova, O. N., Belt, M. L., Mathur, S. P., Lee, J. C., & Akyel, Y. (1996). Effect of Ultrawide Band (UWB) Radiation Pulses on Spontaneous and Ultraviolet Light-Induced Mutation and Mitotic Recombination in Yeast. *Second CERT Symposium for Environmental Radiation Toxicology*.

Pakhomova, O. N., Belt, M. L., Mathur, S. P., Lee, J. C., & Akyel, Y. (1997). Lack of Genetic Effects of Ultrawide-Band Electromagnetic Radiation in Yeast. *Electro and Magnetobiology*, 16(3).

Pakhomova, O. N., Belt, M. L., Mathur, S. P., Lee, J. C., & Akyel, Y. (In press). Ultra-Wide-Band Electromagnetic Radiation Does Not Affect UV-Induced Recombination and Mutagenesis in Yeast. *Bioelectromagnetics*.

Pakhomova, O. N., Mathur, S. P., & Akyel, Y. (1996). Recombinagenic and Mutagenic Processes in Yeast After Exposure to Ultra Wide Band Radiation Pulses. *The Eighteenth Annual Meeting of the Bioelectromagnetics Society*.

- Seaman, R. L., Kiel, J. L., Parker, J. E., Grubbs, T. R., & Prol, H. K. (1996). Effects of Ultra-Wide Band Pulses on Nitric Oxide Production in Murine Macrophages. *The Eighteenth Annual Meeting of the Bioelectromagnetics Society* .
- Seaman, R. L., Kiel, J., & Parker, J. (1996). Ultra-Wide-Band Pulses Increase Nitric Oxide Production in Murine Macrophages. *Second CERT Symposium for Environmental Radiation Toxicology* .

#### **IV. Ocular Effects of High Peak Power Pulsed Microwave Fields**

This very important research project, under the leadership of Microwave Bioeffects Branch is also sponsored by the US Air Force and US Navy's Directed Energy Research Departments. From McKesson BioServices Drs. Shin-Tsu Lu and Satnam Mathur participated as co-principal investigators.

Previous research has reported that pulsed microwave can induce retinal injuries in non-human primates. The retinal injury includes reduction in b-wave amplitude in electroretinogram, cone photoreceptor degeneration (karyolysis and vacuolization), and pyknotic changes of the pigmented epithelium in the monkeys [Kues et al. 1989a, 1989b, 1991, Kues and McLeod 1990, Kues and Monahan 1992]. Increased incidence of chorioretinal scar in "microwave worker" has also been reported [Tengroth and Aurell 1974].

The threshold for pulsed microwave induced retinal injury in primates was considered to be around 4 W/kg. Current personnel protection guidelines are derived from a whole-body average Specific Absorption Rate (SAR) 4 W/kg with a 10 or 50 fold modifying factors for occupational or general population standards. The guidelines allow a local SAR up to 8 W/kg.

Therefore, experimental outcome on the susceptibility of retinal tissue to

radiofrequency induced injury has a direct impact on promulgation of a personnel protection guideline.

The major health implication for the warfighter concerning this issue is that retinal injuries can lead to vision impairment or blindness in severe case. In this experiment the sensitivity of the primate eye to pulsed microwave induced injuries.

Female Rhesus monkeys, 4-5 years of age, 4-5 kg, normal eyes without functional deficit and morphological abnormalities are being used as subjects.

To ascertain normal ocular health prior to exposure and to provide baseline for post-exposure retinal endpoints, the following pre-exposure screening and baselines were obtained: Electroretinogram (ERG) evaluation [Physiological Function], Fundus photographic evaluation [Clinical Morphology], Scanning Laser Ophthalmoscopic (SLO) examination and Fluorescein Angiograph (FA) and Indocyanine Green (ICG) Angiograph [Clinical Morphology].

Pulsed microwaves from a Navy FPS-7 radar operated at 1.25 GHz carrier frequency are used for microwave exposures. An open-ended WR650 waveguide is used as an antenna. The distance between the eyes of the subject and the waveguide is 7.6 cm. The transmitter's peak power is 1 MW and has a pulse duration of 6  $\mu$ s. The pulse repetition rate were 0.6 Hz , 1.2 Hz , and 2.8 Hz



respectively for three dose levels of 4 W/kg, 8 W/kg, and 20 W/kg. Average power densities were 9.5 mW/cm<sup>2</sup>, 19.0 mW/cm<sup>2</sup>, and 47 mW/cm<sup>2</sup> for corresponding dose levels. The subjects were exposed 4 hours/day, 3 days/week, for 3 weeks.

During the post-exposure the following procedures were performed in sequence 24 hours after the last microwave exposure: Post-exposure ERG, post-exposure fundus photographs, post-exposure SLO/FA and ICG angiogram, euthanization, enucleation and histo-pathological evaluations.

Specific absorption rates (SAR) were determined by thermometric dosimetric method using 2 female Rhesus monkey carcasses, at 4.5 and 4.9 kg, respectively. Two Luxtron MAM-05 probes with 4 sensors each and 5 mm spacing were employed via 8 channel Luxtron 3000 Fluoroptic Thermometry System at a 10 Hz acquisition rate and 4 Krohn-Hite 3322 Filters at 1 Hz low pass computer at 2 Hz data acquisition rate via 8 channels. The location of the temperature sensor probes were at 4 quadrants: superior, oral, temporal and nasal along the surface of the globe. Four replications were used for each determination at 4 depths of 0.0, 0.5, 1.0, and 1.5 cm from the Limbus 16 locations per globe and 32 locations for both eyes.

Exposures were performed at 7.6 cm from antenna, vertical E-field,

horizontal H-field, using 1.25 GHz pulsed microwaves at 1 MW peak power and 6  $\mu$ s pulsewidth with 17.5 Hz pulse repetition rate, 80-100 W average power, and a reflection coefficient of  $< 3.4 \%$ .

*Field Characterization:* The L-band exposure system produces 2.79 mW/cm<sup>2</sup> per Watt transmitted through the antenna at 7.6 cm from the antenna where the monkey's head is positioned. The power density decreases exponentially with distance from the antenna at the direction of propagation. The contour of the field is oval. The -3 dB (50 %) field width is approximately 6 cm. The power density decreases to -20 dB (1 %) 15 cm from the center of the field. The exposure is essentially a head-and-neck exposure while anatomical structures below the diaphragm are out of incident field.

*Dosimetry:* The local SAR varies with location and animal. Approximately a 6 fold difference in local SARs are noted. The average retinal SAR is very uniform from animal to animal and eye to eye. The largest difference among 4 eyes is less than 21 %. The average retinal SAR is 1.25 W/kg per W power transmitted through the antenna or 0.45 W/kg per mW/cm<sup>2</sup>.

*Current Status of the Project:* Four monkeys were exposed at each dose level (0, 4, 8, 20 W/kg). The preliminary results indicate that retinal injury did not occur in any of the eleven monkeys examined so far. However, post exposure

effects were noted. Increased glycogen storage in the cone receptors and Muller cells, and enhanced post-exposure cone photoreceptor b-waves were observed. The increased glycogen storage and enhanced b-wave amplitude occurred most frequently in monkeys exposed to 8 W/kg and 20 W/kg but not at 4 W/kg. These effects could be "stressed" photoreceptors which may or may not lead to injury.

#### **Relevant Presentation/Publication**

D'Andrea, J. A., Ziriak, J. M., Lu, S. T., Mathur, S., Merritt, J. H., Johnson, M., Luty, G., Zwick, H., & Stuck, B. (1997). Rhesus Monkey Vision After Exposure to High Peak Power Microwave Pulses. Presented at *The Second World Congress for Electricity and Magnetism in Biology and Medicine* .

D'Andrea, J. A., Ziriak, J., Lu, S. T., Mathur, S., & Merritt, J. H. (1997). Rhesus Monkey Vision After Exposure to High Peak Power Microwave Pulses. In: *Abstracts of the 1997 DOD Electromagnetic Environmental Effects (E3) Program Review* .

## **V. Biological Effects of Millimeter Wave Fields.**

### **A. Genetic Effects Of Low-Level Millimeter Waves**

Most known frequency specific effects of low and extremely-low levels of millimeter waves (MMW) are related to genome structure and function. However, the data have not been generally accepted in the scientific community. The existence of nonthermal genetic effects of MMW requires independent confirmation.

The objective of this research is to investigate possible MMW effects on UV-induced mutagenesis and recombination and to study frequency dependency of MMW effects.

Methods: D7 strain of yeast *Saccharomyces cerevisiae* was used to determine:

- mutations; including reversions, point mutations, deletions, aneuploidy,
- intragenic mitotic recombination (gene conversion),
- intergenic mitotic recombination (crossing-over),
- colony forming ability (CFA).

UV 254 nm (100 J/m<sup>2</sup>) was used as a mutagen. MMW (61.0 - 61.4 GHz, 0.5 mW/cm<sup>2</sup>, 30 min) served as a modifying factor.

The results indicated that MMW at different frequencies enhanced intragenic mitotic recombination. Furthermore, MMW had no effect on mutagenesis, intergenic mitotic recombination or CFA.

Dr. Pakhomova concluded that MMW pre-treatment may change the proportion between pre-mutagenic and pre-recombinagenic DNA damages or enhance the recombinagenic repair process. This implies a possible role of MMW as a carcinogenic or co-carcinogenic factor.

#### **B. Neural Effects of Low-Intensity Millimeter Waves**

Reliable knowledge of frequency specific, nonthermal bioeffects of millimeter waves (MMW) is necessary for development of safety guidelines for human exposure.

The objectives of these studies were: to reveal and analyze specific bioeffects of millimeter waves (MMW) in model nerve and neuron preparations, to develop response spectra with respect to the wavelength and intensity of the radiation, and to define nervous functions and structures most sensitive to MMW.

Models and endpoints employed:

1. Action potential conduction, refractoriness, and tolerance to a high-rate of electrical stimulation in isolated frog sciatic nerve.

2. Synaptic processes in the isolated frog spinal cord, including mono- and polysynaptic conduction via chemical and electrical synapses, pre- and postsynaptic recurrent inhibition, summation on motoneurons, posttetanic potentiation, habituation, heterosynaptic facilitation, etc.

Results indicated that over 200 experiments in isolated nerve and over 60 in isolated spinal cord allowed us to identify significant bioeffects which are induced by MMW intensities far below the effective safety limits. These effects demonstrated resonance-like dependence on the frequency of the radiation, while their dependence on the field intensity was rather flat.

The observed nonthermal, frequency-specific neural effects prove that it is inadequate to base safety standards solely on the thermal action of MMW. Revision of currently effective safety standards requires accumulation of sufficient database on MMW bioeffects and understanding of basic mechanisms of MMW-specific interactions.

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## **VI. Determination of Field Strengths, Dosimetry and Modeling of Radiofrequency Fields.**

### **A. Complex Dielectric Characterization of Materials**

The issue of the lack of complex dielectric data of materials (biological, liquids, solids) at super high frequencies and models in wide frequency band led Dr. Jian-Zhong Bao, to study the "*Complex Dielectric Characterization of Materials*".

The objective of this research was to obtain dielectric spectra and models of biological materials and low and high density solids in a wide frequency range.

To study this problem, we utilized the open ended coaxial probe technique with a computer controlled HP 8510B network analyzer to measure the dielectric spectra in the frequency range between 45 MHz and 26.5 GHz, and the complex nonlinear least squares method to model the dispersions with the common empirical formulas: Debye, Cole Cole, Cole Davidson, Havriliak Negami functions. An object oriented analysis program with C++ has been developed to model the complex dielectric spectra.

Many complex dielectric measurements on biological tissues, tissue simulating phantom material, and some low and high density solids have been performed. The results are consistent with the previously published data in low

frequencies. The data of rat brain tissues have been analyzed extensively.

Thanks to the efforts of McKesson BioServices' scientists, a complex dielectric measurement laboratory has been set up and an object oriented analysis program with C++ has been developed in the Microwave Bioeffects Branch. With these hardware and software tools, it is possible, in principle, to measure and analyze all the liquid and solid materials in the radio and microwave frequencies. This new addition to the capabilities of the US Army's Microwave Bioeffects Branch has been recognized by the other two services on the compound and the facility has been used to provide assistance and support for other Services' needs.

#### **B. Development of Finite-Difference Time-Domain Simulation Software with Object-Oriented Design.**

Lack of effective experimental methods for the measurements of electromagnetic field distributions in a complex system, such as biological bodies, in a detail form is one of the biggest and complex issue in the radio frequency field.

The objective of this project is to develop a generic simulation program using finite-difference time-domain method to study the electromagnetic problems in complex systems. Computer simulation is the only solution to this problem.

The finite-difference time-domain technique is a powerful tool to solve the Maxwell's equation, especially for the complex systems in time domain. With a bigger and faster computer, a better accuracy can be achieved. The Yee's discretization algorithm with Berenger and Mur absorption boundary conditions is utilized.

Developing this software with object-oriented technique offers the following advantages:

- 1) *Code Reuse*: The code is much more apt to be reused again. It is feasible and practical to considering writing something once and to have it used repeatedly.
- 2) *Localization of Change*: Because of the polymorphism and inheritance, changes are usually local to a class rather than spread out across a whole application.
- 3) *Short Development Cycle*: Because of the above advantages, the cycle of development is short, in the long run.

A new Sun Workstation has been acquired for this project. Although Yee's discretization algorithm and some of the boundary conditions have been coded, the code is still under development. In the near future some simple geometry object will be tested first, followed by more complex systems.

### **C. Characterization of Ultra-Wide-Band Electromagnetic Pulses**

The characterization of the ultrawide band exposure system is extremely difficult due to the errors in fast pulse measurements caused by sensors, cables, and a sampling scope. In order to obtain the best characterization of the E-field in the UWB pulses and their energy spectra in an exposure facility for biological effects studies, a two-step deconvolution routine was developed.

The following steps have been taken to correct the measurement errors:

- 1) Obtain the transfer function for the connection cable and SCD5000 system with a pico second pulse generator and a CSA803 sampling scope.
- 2) Model the impulse response function of the D-dot sensor with delta-functions to cancel the reflections, and optimize the parameters of the delta-functions with a reference measurement and our object-oriented software.
- 3) Deconvolve the measured pulse using the transfer functions obtained in the above two steps with various filters.

The UWB pulses delivered by this system have been characterized with this technique and were found to have a rise time less than 166ps and a magnitude as high as 107kV/m. This routine worked successfully in correcting the rise time and the magnitude of UWB pulses, and in compensating pulse shape and energy spectra. This has also been adopted by the Air Force. Four measurements have been performed on their KenTech system thus far.

### Relevant Presentations/Publications

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Lee, J., Bao, J. Z., Lu, S. T., & Seaman, R. L. (1995). An Ultra-Wide-Band Exposure System For Studying Biological Effects. *The Seventeenth Annual Meeting of the Bioelectromagnetics Society*, 160.

## **Microwave Bioeffects Branch Representation in Scientific Meetings**

One or several members of the Microwave Bioeffects Branch staff attended and made presentations at the following meetings:

- ◆ Seventh National Conference on High Power Microwave Technology
- ◆ First, 2nd, and 3rd CERT Symposium on Environmental Radiation Toxicology
- ◆ Second, 3rd., and 4th Annual Michaelson Conference
- ◆ Sixteenth, 17th, and 18th. Annual Meeting of the Bioelectromagnetics Society
- ◆ Fortieth Annual Meeting of the International Society of Optical Engineering
- ◆ Third International Congress of the European Bioelectromagnetics Association
- ◆ Ninth International Congress on Genes, Gene Families, and Isozymes

Besides the above conferences that have been attended, the work of the MBB has also been presented in the following meetings:

- ◆ Second World Congress for Electricity and Magnetism in Biology and Medicine
- ◆ Thirty-Third International Congress of Physiological Sciences
- ◆ Seventy-Third Annual Meeting of the American Association of the Advancement of Science



## CONCLUSIONS

Behavioral, biological, cardiovascular, cellular effects of ultrawide band and high peak power pulsed microwave fields; ocular effects of high peak power pulsed microwave fields, and biological effects of millimeter wave fields were studied using various physiological and behavioral paradigms in animal models. Behavioral studies involved analgesia and open field activity in mice. Cardiovascular effect studies indicated systolic, mean, and diastolic arterial pressures decreased significantly in UWB exposed rats. Cellular effects involved Nitric Oxide Synthase activity in cultured cells, and mutagenic and recombinagenic effects of electromagnetic fields. Millimeter wave studies showed frequency specific neural effects below the effective permissible safety limits. Engineering part of the program involved determination of fields strengths, dosimetry and modeling of radiofrequency fields. A two-step deconvolution routine was developed to characterize ultra fast rise time UWB pulse and has been adopted by other military services to be used in their systems.

McKesson is determined to provide, under the general guidance of the COR and appropriate WRAIR program managers, a high-quality peer-reviewed scientific journal publishable research support. Specific research protocols will focus primarily on the behavioral and biological effects of the electromagnetic radiation in the microwave region of the spectrum. McKesson researchers will continue to investigate potential hazards of the RF Directed Energy Sources to personnel

operating in a tactical environment using existing L-Band, UWB, EMP, and MMW sources supplied by the Government.

McKesson will continue to give priority to behavioral and CNS experiments with a commitment to the highest scientific standards resulting in high quality peer-reviewed publications. We understand that the MMB research efforts in high power microwave fields have little precedent and are considered innovative and pioneering.

We were able to publish and/or present 46 articles over the three base year period. This is excellent proof of the productivity and the dedication of the McKesson staff employed here at The US Army Medical Research Detachment. The value of this productivity is heightened when limited financial resources of the Branch is considered. We do realize the resource difficulties of the Command in recent years. Our staff is willing to accommodate revised and further limited resources and to produce the highest quality research possible under the new circumstances.

With the guidance of the US Army Operational Medicine Research Program managers and our COR Bruce E. Stuck McKesson BioServices scientific staff will investigate new arenas of research to respond to current health and safety issue of the warfighters. We will continue to promote the success of Walter Reed Army

Institute of Research and be the world leader in military relevant biomedical research. We will help WRAIR to ensure that the warfighters will have the best available knowledge and protection against environmental electromagnetic threats. Concurrently, we are committed to support the efforts of the program to acquire new funding from various sources.